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Effects of cyclodextrins on the acid hydrolysis of digoxin

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The effects of three cyclodextrins (α-, β-, γ-CyD) on the acid hydrolysis of digoxin were examined. From the high performance liquid chromatographic tracing of each of the four components (digoxin, bisdigitoxoside, monodigitoxoside, digoxigenin) in reaction mixtures, the individual rate constants (k_1-k_6) were determined by analogue computer simulation. The hydrolysis was suppressed by CyDs in the order of β->γ->α-CyD, where β-CyD inhibited the appearance rates of digoxigenin (k_3, k_5, and k_6) significantly. In the dissolution study of digoxin tablets, the increase in dissolution rate and decrease in acid hydrolysis were attained by inclusion complexation. The data are presented suggesting that CyDs are useful for improving the oral bioavailability of digoxin.

Digoxin, one of the potent cardiac glycosides, is susceptible to hydrolysis in acidic medium, and the therapeutic efficiency as well as oral bioavailability may decrease as a result (Gault et al 1977; Sonobe et al 1980). We have recently reported that the oral absorption of digoxin was significantly improved by cyclodextrin (CyD) complexation (Uekama et al 1981a). In these continuing investigations, the cavity-size effects of three CyDs (internal diameters of 5·0, 6·2, and 7·9 Å for α-, β-, and γ-CyD, respectively, Saenger 1980) on the acid hydrolysis of digoxin were studied in the hope of improving the chemical stability of digitalis glycosides. The release of digoxin from tablet and its simultaneous conversion to hydrolysis products in acidic dissolution medium were also examined and the protective effects of CyDs were discussed on the basis of inclusion complexation (Uekama 1981b). High performance liquid chromatography (h.p.l.c.) was employed to quantitate digoxin and its three potential hydrolysis products; i.e. bisdigitoxoside, monodigitoxoside, and digoxigenin, simultaneously.

MATERIALS AND METHODS

Materials

α-, β-, and γ-CyDs, gifts from Nippon Shokuhin Kakō Ltd., were recrystallized from water. Digoxin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin were purchased from Böehringer Mannheim GMBH. All other materials and solvents were analytical reagent grade. Deionized double-distilled water was used throughout.

Kinetic studies

The parent compound was dissolved in aqueous solution of pH 1·66 and adjusted to an ionic strength of 0·5 M with potassium chloride. The final concentration of substrates in all solutions was approximately $1{\cdot}0 \times 10^{-4}$ M. Five ml of substrate in the absence and presence of $1{\cdot}0 \times 10^{-2}$ M CyDs was incubated at 37 °C. At timed intervals, 100 μl of sample solution were withdrawn with microsyringe and subjected directly to h.p.l.c. analysis.

Determination of rate constants

The individual rate constants for the hydrolysis of digoxin were determined by analogue computer (Hitachi ALS-20M) simulation.† The details of programming and curve-fitting procedure are described by Roberts (1977) and Kurono & Ikeda (1981). The accuracy of the kinetic data was within ± 5%.

Dissolution studies

The release of drug was measured using a rotating disk apparatus (Nogami et al 1966) in 0·05 M KCl-HCl solution (pH 1·52) at 37 °C as described previously (Uekama et al 1982a). The pure digoxin powder (150 mg, 100 mesh) was compressed into cylindrical tablets (diameter 10 mm) at a pressure of about 200kg cm^{-2}. At appropriate intervals, 1 ml of aqueous solution was removed from the flask and extracted with chloroform. After evaporation to dryness and appropriate dilution with methanol, the sample solutions were analysed by h.p.l.c. Correc-

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† The details of the programming and procedure are available from the authors on request.

tions were applied for cumulative dilution caused by replacement of sample by equal volumes of the original medium. The tablets maintained a constant shape throughout the measurement. A solid complex of digoxin—γ-CyD complex (1:4 molar ratio) was prepared as described previously (Uekama et al 1982b) and its dissolution behaviour in acidic medium was examined similarly.

H.p.l.c. analysis

For the separate determination of digoxin and its hydrolysis products, the method of Sternson et al (1978) was used. The chromatograph (ATTO HSLC-013-4) was operated at flow rate of 0·7 ml min^{-1}, and the eluent was monitored spectrophotometrically at 220 nm. The separation utilized a column, LiChrosorb RP-18 (5 μm in 4 mm × 25 cm, Merck), with methanol–water (58:42) as a mobile phase. Components were quantitated by measuring peak heights and comparing the height with that of known amounts of internal standard, n-butyl *p*-hydroxybenzoate.

RESULTS AND DISCUSSION

Acid hydrolysis of digoxin (D) is known to be a complex combination of parallel reactions (Scheme 1), where the disappearance of D and appearance of hydrolysis products; bisdigitoxoside (B), monodigitoxoside (M), and digoxigenin (G); can be described as pseudo-first-order process (Sternson et al 1978), expressed by:

$$-\frac{dD}{dt} = (k_1 + k_2 + k_3)D \quad (1)$$

$$\frac{dB}{dt} = k_1D - (k_4 + k_5)B \quad (2)$$

$$\frac{dM}{dt} = k_2D + k_4B - k_6M \quad (3)$$

$$\frac{dG}{dt} = k_3D + k_5B + k_6M \quad (4)$$

In the degradation pathways of digoxin, prevention of the appearance of digoxigenin might be clinically important because the cardioactivity of digoxigenin is about one-tenth of that of digoxin but other glycosides possess approximately the same activity (Kroneberg 1959). In the present study, the effects of three CyDs on the hydrolysis of digoxin were studied, since it forms inclusion complexes with CyDs in aqueous solution (Uekama et al 1982b). Taking into account the magnitudes of the stability constants obtained by the solubility method (Uekama et al 1982b: 180 M^{-1} for the α-CyD complex, 11200 M^{-1} for the β-CyD complex, 12200 M^{-1} for the γ-CyD complex), the concentration of CyDs ($1{\cdot}0 \times 10^{-2}$ M) was employed largely in excess compared with that of substrates ($1{\cdot}0 \times 10^{-4}$ M). Relatively low pH (1·5–1·7) and temperature of 37 °C were chosen to accelerate the reaction rate because the measurement was kinetically convenient.

Fig. 1 shows a typical example of the variation in the composition of digoxin hydrolysis as a function of time at pH 1·66. Each hydrolysis product in the absence and presence of CyDs in hydrolysates was expressed as the mole fraction of all digoxin species present. The disappearance of digoxin and appear-

Scheme 1

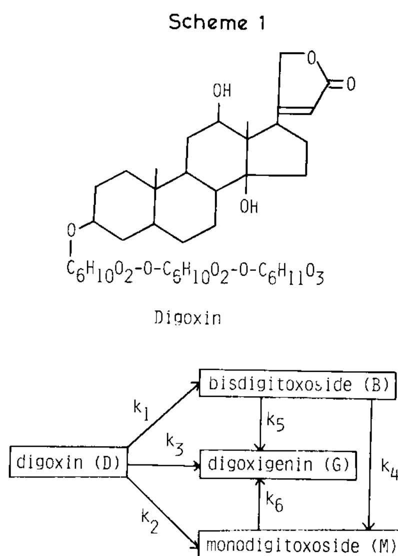

Table 1. Rate constants (h^{-1})[a] for digoxin hydrolysis in the absence and presence of CyDs (1×10^{-2} M) at pH 1·66, 37 °C.

Constant	Without CyD	With α-CyD	With β-CyD	With γ-CyD
k_1	0·376	0·340	0·338	0·345
k_2	0·146	0·130	0·101	0·118
k_3	0·171	0·108	0·002	0·025
k_4	0·325	0·288	0·255	0·295
k_5	0·189	0·070	0·005	0·020
k_6	0·285	0·128	0·002	0·056
k_M[b]	0·285	0·128	0·003	0·056
k_B[b]	0·514	0·356	0·262	0·333
k_D[b]	0·641	0·498	0·398	0·433

[a] Accuracy of ±5%.
[b] Determined experimentally from disappearance of digoxin species.

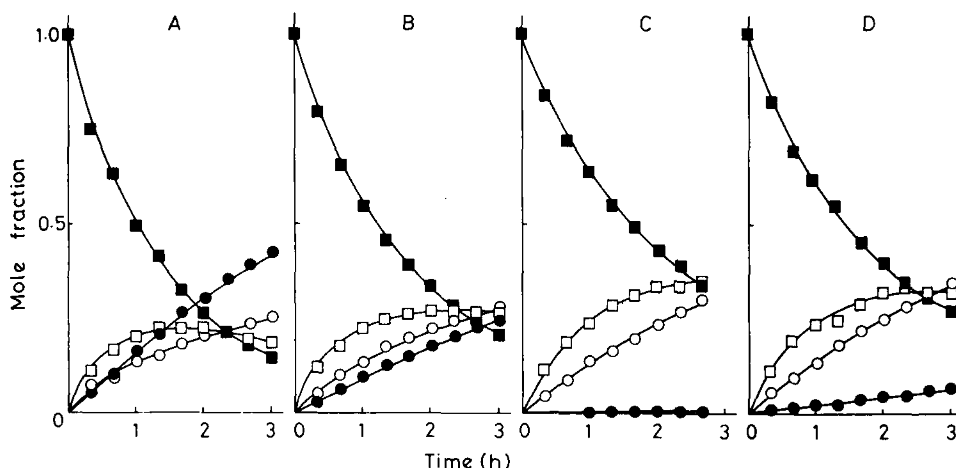

FIG. 1. Time-conversion profiles of digoxin species present in digoxin solution at pH 1·66, 37 °C. (A): digoxin alone. (B): digoxin—α-CyD system. (C): digoxin—β-CyD system. (D): digoxin—γ-CyD system. ■: digoxin. □: digoxigenin bisdigitoxoside. ○: digoxigenin monodigitoxoside. ●: digoxigenin. Curves were traced by the computer using the individual rate constants listed in Table 1.

ance of three hydrolysis products (B, M, G) were found to be pseudo-first-order in all cases. As shown in Fig. 1, the hydrolysis of digoxin was suppressed by the CyDs. To determine which reaction pathways in Scheme 1 were affected by CyDs, the individual rate constants (k_1–k_6) were estimated by means of the analogue computer simulation. In a series of parallel experiments, the acid hydrolysis of reactive substrates (B and M) similarly examined. Table 1 summarizes the rate constants determined for hydrolysis of various digoxin species in the absence and presence of three CyDs ($1 \cdot 0 \times 10^{-2}$ M). Under present experimental conditions, the rate constants for CyD systems may be taken as those for complexes, because the stability constants of inclusion complexes are relatively large. The accuracy of data is demonstrated by comparison of k_B and k_M, determined experimentally for hydrolysis of bisdigitoxoside and monodigitoxoside, with the individual rate constants, ($k_4 + k_5$) and k_6, respectively. The

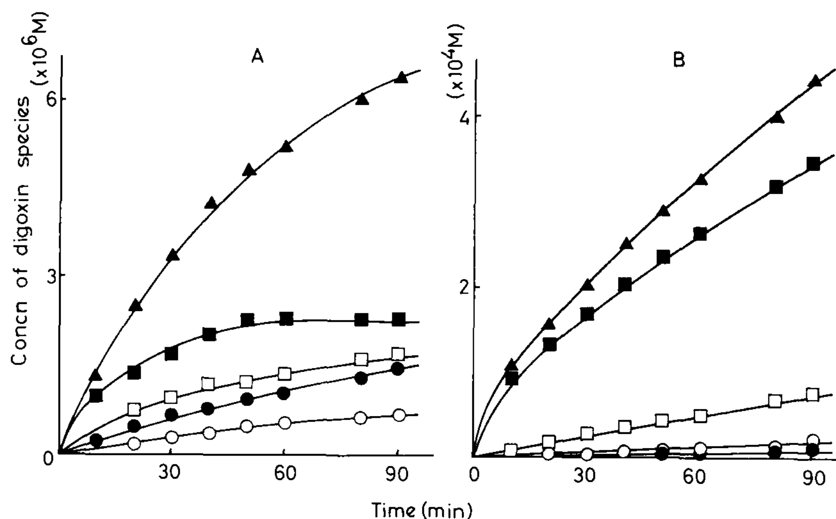

FIG. 2. Release and degradation profiles of digoxin or its γ-CyD complex following the dissolution from compressed tablets in acidic medium (pH 1·52) at 37 °C, measured by the rotating disk method. (A): digoxin alone. (B): digoxin—γ-CyD complex. ▲: total amounts of digoxin species. ■: digoxin. □: digoxigenin bisdigitoxoside. ○: digoxigenin monodigitoxoside. ●: digoxigenin.

goodness of fit is also shown by comparing the pseudo-first-order rate constant (k_D), determined experimentally for digoxin disappearance, with the sum of individual rate constants ($k_1 + k_2 + k_3$) determined by curve-fitting procedure. It is apparent that the inhibitory effects of CyDs on the degradations of digoxin species were generally in the order of β->γ->α-CyD. These results indicate that either a smaller (α-CyD) or a larger (γ-CyD) cavity is unfavourable for preventing the acid hydrolysis of digoxin. In fact, our previous ^{1}H-n.m.r. data suggested that the A-ring moiety of digoxin molecule is located at the entrance of the α-CyD cavity, it could penetrate further into the β-CyD cavity, and is loosely bound to γ-CyD (Uekama et al 1982b). β-CyD inhibited the conversion of digitoxosides to digoxigenin (k_3, k_5, and k_6) almost completely. This finding may be particularly useful from the view point of oral digoxin therapy.

Since the solid complex of digoxin with γ-CyD was obtained (Uekama et al 1982b), the release of digoxin and its simultaneous conversion to hydrolysis products from the complex were compared with those from digoxin itself. Fig. 2 shows the comparative degradation profiles following the dissolution from compressed tablets of digoxin and its γ-CyD complex in acidic medium (pH 1·52) at 37 °C. In the case of digoxin tablet, the release of intact digoxin reached a peak in 60 min, and was then followed by a gradual decrease owing to the appearance of hydrolysis products. On the other hand, the complexed form of digoxin dissolved much more rapidly (about 100-fold) than digoxin itself, which may be due to the increase in solubility and/or the decrease in particle size by inclusion complexation. Although a higher release of digoxin was attained by γ-CyD, the hydrolysis was significantly suppressed and in particular the appearance of digoxigenin was almost negligible even 60 min after the initiation of the dissolution test.

These limited data suggest that variation in oral bioavailability of digoxin owing to poor dissolution and acid hydrolysis in stomach may be improved by CyD complexation, without lowering the pharmacological activity.

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